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The life history of Cronartium coleosporioides

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THE LIFE HISTORY OF CRONARTIUM COLEOSPORIOIDES

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INTRODUCTION

Within the past few years much has been disclosed by various workers regarding the life history of the rust Cronartium coleosporioides (D. & H.) Arth. attacking certain of the hard or yellow pines. Cultures and field observations have aided in establishing the alternate hosts to be species of Castilleja, Orthocarpus, and Pedicularis. The pycnial stage has been found and studied and many other points of interest have been brought out in the course of the work done in this region. An attempt is here made to combine all the information already published with further information secured by field observations and by cultural and microscopic work, in an endeavor to present in detail the complete life history of this interesting rust. Since some uredinologists believe that the two principal and somewhat distinct forms of hypertrophy assigned to this rust are either distinct species or are races of the same fungus, considerable attention will be given in this paper to this phase of its life history.

DISTRIBUTION

The distribution of this rust is decidedly Western. It is found in all the mountainous regions where its hosts and alternate hosts are commonly found, extending throughout the Rocky Mountain and Pacific Coast regions from the eastern slopes of the Rockies to the Pacific Coast and from the Mexican border up into Canada. So far, it has been collected in Washington, Oregon, California, Nevada, Colorado, Arizona, Wyoming, Idaho, and Montana.

A graphic representation of the distribution by states of the rust on its various hosts, in so far as collections, reports, and field observations indicate, is given in the outline map (fig. 1).

The rust is common in two types of forest in this region, the lodgepole pine (Pinus contorta) type and the yellow pine type (Pinus ponderosa) type, following both across the northern border into Canada.

LIFE CYCLE OF THE RUST

Hosts and Alternate Hosts

The life cycle of this fungus necessitates its existing part of the time upon the cortex of the hard or yellow pines and the remaining interval upon species of Castilleja, Orthocarpus, and Pedicularis. The pycnial and aecial stages develop upon species of the genus Pinus while the uredinial and telial stages parasitize Scrophulariaceous plants.

The pycnial stage (fig. 2) appearing on both ruptured and unruptured areas of the hypertrophied tissues has been collected upon two hosts; viz., *P. ponderosa* and *P. contorta*.[36]

The aecial stage (figs. 3, 4, and 5.) has been collected on the following hosts: *P. ponderosa*, *P. scopulorum*, *P. contorta*, *P. jeffreyi*, *P. murrayana*, *P. coulteri*, *P. attenuata*, and in the Northwest, on *P. ponderosa* and *P. contorta* only (figs. 3, 4, and 7).

Until quite recently the only hosts upon which the uredinial and telial stages (fig. 6) were found were species of Castilleja. Bethel in 1915[38] and Bethel and

Hunt in 1917[38] collected the rust on species of Orthocarpus in Colorado. Weir and Hubert[38] reported a collection of the rust on *Pedicularis groenlandica* made by F. S. Wolpert at Big Springs, Idaho. Uredinial and telial stages are now reported on the following hosts: *Castilleja foliolosa*, *C. miniata*, *C. linearis*, *C. angustifolium*, *Orthocarpus luteus*, *O. purpureo-albus*, and *Pedicularis groenlandica*.

Description of the Four Stages

Technical descriptions of the various stages of the fungus under discussion might prove of value here, in order to definitely establish its characteristics before going on with a discussion of the cultures and other phases of the work. The description of the pycnial stage is based on material taken from the gall type of infection. Up to the present time the pycnial stage for the typical *P. stalactiforme* type and its variations has not been demonstrated. A few additional characters have been added to the description of the I stage.

Cronartium coleosporioides (D.& H.) Arth.[1]
Uredo coleosporioides Dietel and Holway.[5]

O. Pycnial stroma in irregularly shaped areas, more or less scattered or anastomosing, caulicolous, subepidermal, forming minute, blister-like swellings when mature on unruptured infected tissues and issuing from cracks in the bark of old lesions; exuding a clear, sweet, sticky fluid in which the pycnospores are suspended, forming drops of a cadmium yellow to orange color when first appearing, becoming clear as the spore mass settles to the lower end of drop, and orange to brick-red upon drying. Pycnospores hyaline, mostly spherical, occasionally ellipsoid or obovate. (5) 1.5 to 3.0μ by 1.5 to 3.7μ (2.5 by 2.5).

I. Aecia occurring on fusiform and globoid galls and slight swellings of branches and twigs, on cankers and slight swellings of the trunk, occasionally confluent, cylindrical, subcompressed to hemispherical, depending on resistance and condition of bark, 1 - 2 mm. in diameter and 1 or 2 to 7 or 9 mm. high; peridium rupturing apically, occasionally laterally, edges fringed, with evident more or less numerous filaments extending from base to apex of sorus, these measuring 0.5 - 8.5 mm. long and 0.15 - 0.35 mm. wide at base, tapering slightly toward apex;

aeciospores obovate-oblong, or ellipsoid, 14-24 by 23-35μ; wall 2.5-4.0μ thick, closely and coarsely verrucose, some spores showing a smooth area near base.

II. Uredinia hypophyllous and caulicolous, rather crowded in groups 1-5 mm. across, round, minute, 0.1 mm. across or less, dehiscent by a small central opening; peridium delicate; urediniospores globoid or broadly elliptical, 14-22 by 17-27μ; wall colorless, thin, 1-1.5μ, sparsely and very minutely echinulate.

III. Telial columns hypophyllous, cylindrical, short, 0.5 mm. long, 80-110μ thick; teliospores oblong or fusiform-oblong, 12-17 by 30-52μ, obtuse at both ends; wall nearly colorless, smooth, thin, 1μ.

LIFE CYCLE

Review of Cultures

The aecial stage on the branches and twigs of *P. ponderosa* was the first stage found and described. This was done by Peck in 1882[28], who gave it the name

Peridermium filamentosum on account of the numerous filamentous processes found within the peridia. These filaments are very long and very characteristic in the type material (Portion of type, No. 98*) and the peridia are remarkably elongated and cylindriform (fig. 5). The type material is the only material so far collected which possesses these extreme characters. This, coupled with the fact that the peridial walls, with the exception of the upper portion, are composed of only one layer of cells instead of two, indicates the type specimen to be an abnormal representative of the species.

The uredinial and telial stages developing on Scrophulariaceous plants was first described by Dietel and Holway[6] in 1893 as *Uredo coleosporioides* (D. & H.). In 1907 Arthur[1] re-described it as *Cronartium coleosporioides* (D. & H.) Arth. These stages of the rust were first collected on *Castilleja foliolosa* at Berkeley, California, and have subsequently been found on other species of Castilleja as well as upon closely related genera.

In 1916[36] the pycnial stage (fig. 2) was demonstrated by Weir and Hubert, who secured abundant pycnial

*Collections at Missoula, Mont.

exudations from typical galls collected on *P. ponderosa* and *P. contorta.*

A review of the cultures of this fungus briefly presented, will serve to keep the points in mind which will later be presented as partial proof of the identy of the gall form with the stalactiform type of hypertrophy.

In June of 1912, Meinecke[26] successfully sowed aeciospores taken from *P. stalactiforme* on several plants of *Castilleja miniata* and determined *P. stalactiforme* to be the aecial form of *Cronartium coleosporioides.* Hedgcock[15] in July of 1912 with aeciospores taken from a semi-typical form of *P. filamentosum* secured successful cultures on species of Castilleja. On account of Meinecke's[26] reporting successful cultures on Castilleja with typical *P. stalactiforme* and naming it *C. coleosporioides,* Hedgcock gives the fungus he has cultured a new name, viz., *C. filamentosum* (Pk.) Hedg. Hedgcock and Long[17] in June, 1913, successfully cultured aeciospores taken from typical P. *stalactiforme* on *P. contorta* upon *Castilleja linearis.* The next cultures were conducted by Weir and Hubert[35] in May, 1915, who successfully cultured the rust on species of Castilleja by sowing aeciospores taken from infections on seedlings of *P. ponderosa*, "hip canker" (fig. 7) and gall forms (fig. 3) of *P. contorta* and from typical *P. stalactiforme* (figs. 4 and 11) on *P. contorta*. All the forms above mentioned were included under the name *P. filamentosum* representing the aecial stage of *C. coleosporioides*. Later in May and June of 1916 the same writers[27] carried out a series of cultural experiments in order to check previous cultures. Results were obtained on species of Castilleja by sowing aeciospores taken from the typical stalactiform type on *P. contorta*, typical gall form on *P. contorta* and *P. ponderosa* and from the slightly hypertrophied infections on the stems of young *P. ponderosa* seedlings.

This review of cultures shows that both Meinecke and Hedgcock, working with infections on pines having little or no hypertrophy, secured their results on the same alternate hosts, viz., species of Castilleja. Since there is only one Cronartium described as infecting Castilleja, and since Arthur and Kern[2] state they can find no differences in the gross and microscopical characters of the Cronartium found on Castilleja and collected from every part of its geographical range, it appears that another Cronartium must be described in order to hold *Cronartium filamentosum* as a legitimate species. Hedgcock and Long[20] state that "*Peridermium filamentosum* on *Pinus ponderosa* and *Pinus contorta* is the aecial stage of *Cronartium filamentosum* (Pk.) Hedg., which attacks a number of species of *Castilleja* in the western United States over a wide region, ranging from the Rocky Mountains to the Pacific Coast, *Peridermium stalactiforme* (Arth. & Kern) and *Cronartium coleosporioides* (D. & H.) Arth. are synonymous with this species." The cultures cited add evidence to the tenure that the various forms of the rust discussed in this paper are but variational developments of an identical fungus, *Cronartium coleosporioides.*

Peridermium harknessii Moore, described by Harkness[12] in 1884, closely resembles in the given description the gall-forming fungus on *P. contorta* and *P. ponderosa* of the Montana and Idaho regions, although in the latter regions no oaks are to be found as possible alternate hosts. A close study of the literature shows that *P. harknessii* is proving rather puzzling in its host relationships and is found to be tentatively included under *Cronartium cerebrum* (Pk.) H. & L. (*Peridermium cerebrum* (Pk.)) by Hedgcock[14], Hedgcock and Long[19], Arthur and Kern[2], while Meinecke[27] finds the same fungus to be facultative (?)[9] heteroecious on both the coniferous and broadleaf hosts in the California region. Arthur and Kern include the gall forms on P. murrayana (P. contorta) collected in the Rocky Mountains and in Montana under P. cerebrum, stating that "the Colorado and Montana specimens are included here on morphological grounds, although some doubt is thrown upon this disposition by the failure up to this time to find the alternate stage within this geographical range."

Bethel in a letter of April 6, 1917, states that he has made many cultures of P. harknessii, so-called, on species of Castilleja in the open and has not been able to differentiate the telial stage of this result from telia of the typical P. filamentosum inoculations on the same herbaceous hosts.

Massee[25] holds that P. harknessii Moore is identical with P. filamentosum Pk. and illustrates both the typical gall hypertrophy on an eight-year-old stem of P. ponderosa and a slight hypertrophy on a three-year old stem under the name Peridermium harknessii. A cross-section shown of the gall indicates the infection to have occurred when the twig was two years old.

The literature indicates that neither Hedgcock[14,16,18] nor Meinecke[27] tried to culture aeciospores of _P. harknessii_ on species of Castilleja, while both secured negative results on species of Quercus. There is a possibility that the name _P. harknessii_ has been covering a typical gall-forming rust (probably _P. cerebrum_) which goes to oaks, and also a typical gall-forming species (_P. filamentosum_) (fig. 3) which goes to Scrophulariaceous plants.

The Periods of Development of the Various Stages

The period of development of this rust from the time of infection on pines by means of sporidia from the telial stage to the development of aecia, and thence the transfer to the herbaceous host again has not been thoroughly demonstrated. The perplexing variations found in the period of development on the pine hosts for the two principal types of hypertrophy makes the problem at first glance appear difficult.

The pycnial stage of the gall type of malformation has been described[36] as developing on both unruptured and ruptured infected tissues. Such galls as were found not previously ruptured by the formation of aecia and bearing pycnia were found to range from two to three years in age, judging from the annual rings infected. Young galls bearing their first development of aecia, when sectioned and examined, showed the number of years required to develop the aecia from the time of first infection to be from two to five years. It appears that under unfavorable conditions a gall will continue to develop in size from year to year without fruiting, the mycelium in the tissues remaining active during this period and finally producing fruiting bodies. Again in other cases the galls have developed only two years from the time of infection and at the beginning of the third year produced aecia in abundance. The shortest period of development for aecia is found in the case of the two-year-old P. ponderosa seedlings at Haugan, Montana, infected in the crowded nursery beds[35]. In this case the seedlings developed from seeds in the spring of 1913 and during the same summer were presumably inoculated by sporidia from the III stage on Castilleja. In the spring of 1914 a small percentage of the seedlings developed mature aecia, giving a period of eleven or twelve months from the time of infection to the time of first fruiting. During May of 1915 a larger number of the seedlings developed aecia, and these were produced on slight fusiform swellings on the lower stems. This irregularity in the development period parallels in a measure a similar irregularity of the gall type of infection on the native host trees surrounding the nursery and of the Northwest region in general. The pycnial stage has been found to develop early in the season, about April or May. It has been produced in the laboratory as early as March, by a forcing process. This stage has been difficult to find in the field, which may be due to the fact that the pycnial drops (fig.2) dry out very rapidly and the crusted residue left is scarcely distinguishable from the bark. The pycnial drops develop readily and become quite large whenever the infected portions of the host are kept in moisture-laden air. The finding of the pycnial drops on lesions in the early mornings may throw some light upon the question, for it may be that the drops develop only during moist nights and dry rapidly at the approach of sunlight.

The aecia usually follow the pycnia in the same season, but on different areas of the lesion, appearing from eight to sixteen days after the pycnial drops have formed[35]. Spaulding[30] has observed that the pycnia precede the aecia by a short period in the case of *Cronartium ribicola*. Hedgcock and Long[19] find that in the case of *Peridermium cerebrum* on *Pinus virginiana* the pycnia precede the aecia by twelve months instead of preceding them during the same season. The aecia of *C. coleosporioides* mature and shed their spores from May to October, and in a few rare cases collections have been made in March and in December during chinook spells of warm weather. During the early spring the herbaceous host plants are developing their new shoots, and infection by means of aeciospores (fig. 8) takes place. During the period from the latter part of May to early September the uredinial and telial stages appear on the leaves, stems, and flowers of the herbaceous host. The uredinial pustules (figs. 6 and 9) develop first as minute, hemispherical, orange-colored bodies on either leaf surface. These produce urediniospores which spread the infection on the herbaceous host during the summer. The telia (fig. 9) as cylindrical, partly curved columns at first arise from the uredinia and later develop independently upon the infected leaf. In the field, uredinia appear upon Castillejas about fourteen to twenty days following the first eruptions of the nearby aecia on the pines. The telia begin to appear soon after and continue to develop until late in the summer.

Of the cultures on Castilleja by aeciospores from P. stalactiforme secured by Meinecke, Hedgcock, and Hedgcock and Long, the first by Meinecke developed uredinia and telia in seventeen days, and the next by Hedgcock and Long developed uredinia in eleven days, with telia in twenty-two days. This gives an average for uredinia of thirteen days. Of the cultures made by Weir and Hubert, those made with aeciospores of the stalactiform type, including the infections on the seedlings, give a range of from twelve to twenty-four days, and an average of nineteen days for the development of uredinia; and a range of from fifteen to twenty-eight days and an average of twenty-three days for telia. Of the cultures secured from aeciospores of the gall type, the average of development for uredinia is twenty-three days, and the range from 18-28 days, and for telia the average is 30 days, the range being from 26-38 days.

VARIOUS FORMS OF THE RUST ON CONIFERS

The grouping under one species[35], of a rust developing upon two distinct hypertrophies occurring on trunks and branches of pine has caused considerable discussion among uredinologists and has created new interest in the caulicolous rusts on Pinus in the Northwestern region. At best the determination of species of Peridermium is difficult and in most cases must depend primarily upon inoculation results. The determination of these species by means of spore measurements alone does not seem a reasonable and dependable method, in view of the possible variations in the development of the spores. This fact has led to a comparison of the various characters of Cronartium coleosporioides (D. & H.) Arth. (P. filamentosum Pk.) (P. stalactiforme) as it occurs on its coniferous hosts, with special reference to the occurrence and probable function of the filaments found within the aecia. Pedigree cultures of these rust forms are in progress, and these will no doubt furnish conclusive evidence of their taxonomic position.

Until recently the literature dealing with the descriptions of Peridermium species has shown unmistakable tendencies toward treating and classifying them more or less according to the particular shape which the hypertrophy happened to assume. The assumption being that each species produced a distinct and characteristic swelling of the portion of the host attacked. Recently[19] it has been determined, in one case other than the one in question, that the same species of Peridermium (Cronartium cerebrum (Pk.) H. & L.) is capable of producing at least two entirely distinct abnormalities. This decision has been reversed (1918) by Hedgcock, and Hunt[22b], who claim that pedigree cultures demonstrate that P. fusiforme and P. cerebrum are distinct species. These variations in the form of hypertrophy are in part due to the varying reactions of the host tissue to the fungous attack. Arthur and Kern[2] make the statement that the form of the gall may be dependent upon the rate of growth in the affected part at the time of infection and for some time thereafter. Observations made upon the three principal forms of hypertrophy (globoid (figs. 2 and 3), stalactiform or slightly swollen (figs. 4, 10, and 11), and canker (fig. 7)) occurring on lodgepole pine (Pinus contorta Loud.) seem to indicate that the age and condition of the cellular tissue attacked are in a great measure responsible for these results. The "hip canker" is a hypertrophy of the trunk having a flat, indented face of dead and infected tissues on one side of the stem with two corresponding bulges of the tissue on either side of this. The cankered area which dies out in the center is located in the indented portion and the hypertrophied tissues on either side carrying a large portion of the active fungus, give a bulging effect to the whole. This may be identical with "cat face" as applied to Peridermium cankers in other regions. Spheroid galls are often the origin of such cankers.

A careful examination of the aecia of many specimens (taken from the same tree whenever possible) showed a close morphological resemblance in all cases. The shape, size, and habit of the peridia, the size, form, and markings of the spores, and the common occurrence within the peridia of the characteristic filaments (Figs. 12, 13, and 14), all coincide closely with the description of the aecial stage of the fungus as given by Arthur and Kern[11] and with the actual observations of a portion of the type material, with two exceptions, the unusually long peridia of the type material (Fig. 5) and the number of cell layers in the peridia walls. These observations are arranged in tabular form in Table I and include for comparison C. coleosporioides on Pinus ponderosa.

le I.-- Comparison of Peridia, Aeciospores, and Filaments.
Cronartium coleosporioides (D. & H.) Arth.

t and form of pertrophy	Locality	Peridia Diameter mm.	Height mm.	Shape	Method of rupture	Thickness
us con- rta-- nker on unk.	Haugan, Mont. Coeur d'Alene, Idaho, Wise River, Mont			Short cylindrical, globoid to hemispherical.	Apical, occasionally laterally fringed or toothed edges.	2 cells
us con- rta. oboid gall	Haugan, Mont. Wise River, Mont Coeur d'Alene, Idaho			Do.	Do.	2 cells
us con- rta. siform to one.	Haugan, Mont. Spokane, Wash.	1.5-9 by 1.7-7	9.7-3.0	Do.	Do.	2 cells
us pond- rosa. oboid.	Coeur d'Alene Idaho.			Do.	Do.	2 cells
us pond- rosa seed- ngs(slight siform) em blisters	Haugan, Mont.	1.5-2 by 2.0-3.5		Do.	Do.	2 cells
[illegible] rosa [illegible] ell [illegible] rtion [illegible] pe [illegible]	[illegible]	1.5-2.1	3.0-7.5	Very long, cylindrical; rarely globoid or hemispherical.	Apical, slightly fringed edge on small aecia.	2 cells in upper part; 1 cell in lower part.
us con- orta. stalacti- forme type)	Collected by Meinecke. Cal.	1.5-2.	2.0-3.5	Short, cylindrical. Globoid to hemispherical.	Apical, slightly fringed edge, sometimes lateral.	2 cells

	Aeciospores		Filaments							
[illegible]	Shape	Markings	L'ght full sized fila-ments mm.	Width of base mm.	Range of num-bers in aecia	Av. No. in aecia	Total No. of fila-ments	Size of cells µ	No. of cells thick at base	Basis No. of aecia
24 - 67	Obovate oblong, rarely ellipsoid	Closely and rather coarsely verrucose			4-31	12	722		3 to 5	60
	Do.	Do.	0.5 to 2.5	0.15 to 0.25	1-20	8	478	52-85.5 by 14-26	3 to 6	60
	Do.	Do.	0.5 to 1.5	0.2 to 0.3	2-18	8	483		3 to 5	60
28 36	Do.	Do.	0.5 to 2.6	0.2 to 0.3	1-17	7	348	54-81 by 13-25	3 to 7	50
	Do.	Do.	0.4 to 2.7		1-16	6	371		3 to 5	60
4 6	Obovate oblong, ellipsoid	Closely coarsely verrucose	1.5 to 8.5	0.2 to 0.35	3-26	12	182	59-75 by 14-21	4 to 7	15
7 8	Obvate oblong	Do.	1.5 to 3.0	0.2 to 0.25	4-19	11	215*	54-73 by 12-22	3 to 5	20

*Large number attached to roof of peridia.

the canker forms. This has been brought out by a systematic comparison of counts made from the different hosts and forms as given in Table I under the main division "filaments." To supplement this, similar measurements were made on a portion of the original material of Peck's P. filamentosum (fig. 5) kindly sent by House of New York. This is also included in Table I.

The stalactiform lesions of P. contorta support aecia having short processes extending from the dome of the peridium and somewhat longer ones from the floor. This bears out Arthur and Kern's[2] observations on P. stalactiforme, of which the fusiform lesions examined are typical.

The filaments are very easily distinguished in all fresh specimens by the aid of a hand lens, and occur in a large majority of the aecia (figs. 4 and 14). Those protruding full length from the base of the peridium measure from 0.4 mm. to 3.0 mm. long and 0.15 mm. to 0.30 mm. wide at the base. They are generally broader at the base where attached to the floor and taper somewhat toward the end attached to the dome (figs. 12, 13, and 14), there broadening out slightly in forming an upper attachment.

In many cases these processes extend downward in varying lengths from the dome of the peridium with the remainder of the filament extending upward from the floor. Occasionally the full length extends downward from the dome and often in removing the flaky upper portion of the peridium all the filaments were found to remain attached to it. The rupture of these filaments takes place at the time of opening of the aecia, as has been demonstrated by removing a portion of the peridium of a nearly mature aecium and carefully extracting the spores from under the covering. This disclosed the filamentous processes extending continuously from the floor to the dome of the fruiting body (figs. 13 and 14). Many of the partly matured and ruptured aecia were observed to contain these filaments unbroken. In such cases the processes were generally found attached considerably to either side of the central apical line of rupture of the peridium. As a rule the majority of filaments are found along the median line corresponding to the line of rupture.

The filaments are composed of elongated irregularly compressed cells similar in structure to the cells of the peridium (fig. 15). A peculiar compression of one end is common, which is sometimes drawn out and almost pointed. These cells lie with their long axes parallel to the long axis of the filament, the pointed or narrowed end toward the apex of the filament, and upon drying readily separate from each other. They measure from 52 to 85µ long and 12 to 26 µ wide, and have characteristic, coarse varicose markings over the walls. The filaments are from 4 to 6 cells thick at the base.

From these observations it is to be inferred that the filaments serve as retention elements for the peridium, reinforcing the dome against the pressure of the fast-increasing spores within. When this pressure has reached a certain point and the upper layers of spores are mature and ready to escape,,the retention filaments rupture at right angles to and at varying lengths along their axes. This rupture takes place presumably at their weakest points. This action leaves the dome of the peridium without a restraining force,and, due to the increasing pressure of the spore mass within, it ruptures apically with an uneven tear along its broadest diameter, forming a fringed, spiny or toothed edge (fig. 14). In some cases the peridium is ruptured along its side walls as well.

It is noted that the general method of rupture varies radically from that given for this species. An apical rupture giving fringed, irregular edges is found to be common, instead of a lateral rupture with no special mention of the edges, as has been recorded by Arthur and Kern[2]. It is surprising that the remarkably striking fringed or spiny characteristic has not been noted earlier than by House[23] in his note on P. cerebrum Pk. and Clinton[4] on C. comptoniae. From the photograph given by House of the fungus on a cone of Pinus chihuahuana, and also from actual observation of the specimen kindly loaned to the laboratory by House, it seems that the aeoia do not disclose a lateral method of rupture. They are open at the centers of the apexes, and each appears to have ruptured centrally at the apex of the peridium. Clinton[4], in describing Cronartium comptoniae Arth., mentions the distinct "interlocking teeth or spiny processes" of the peridia, of which his photograph (Pl. XXVIII, fig. b) gives a good representation. The majority of these peridia also appear to have ruptured more or less apically. Weir[34], in connection with P. comptoniae on P. divaricata, mentions the peridia having spiny margins, and both his figure (fig.4) and the original material indicate an apical rupture to be more general than the lateral method. Recent collections of _P. comptoniae_ show this fringed edge of the ruptured aecia as well as the presence of abundant filaments in the peridia. The long cylindrical aecia of the New York material do not possess the fringed edges, but appear to rupture more or less apically. The smaller, short, and globoid aecia appear to have fringed edges, although it is difficult to state positively in this respect, since the material may have become broken. The rupture appears to be apical, but here again it is difficult to determine. The remarkably long aecia of the type material (fig. 5) when removed from the host have the appearance and shape of a hound's incisor and are literally crowded with the long continuous filaments. The shorter, smaller aecia have fewer filaments and of a much shorter length. The filament cells (fig. 16) very closely resemble those of the other types compared. The oblong, narrowed cells (fig. 15) somewhat pointed at times and slightly curved at one end, are present in all the filaments examined, as are the characteristic coarsely verrucose markings. Occasionally broader cells are found, but even these show the compressed or pointed character at one end, due to the method of cell arrangement in the filament. This arrangement, as in all filaments examined, is a typical overlapping one (figs. 15 and 16). The lower portions of the peridial walls appear to be made up of one layer of cells only. This indicates an abnormal condition to begin with.

A study of Table I will show a few interesting facts concerning the relation between the form of hypertrophy and the size and shape of the aecia. The infections of young stems and branches producing little or no swellings seem to possess longer and more cylindrical peridia, and at the same time more and longer filaments. The type material seems to be of this class, and a specimen of Pinus contorta infected with P. filamentosum, collected in 1917 (No. 104), upon examination approached quite closely in habit, size, and other characteristics the type material even to unusually elongated peridia. Infections occurring on the cankers and globoid galls seem to possess a shorter, cupate habit with the aecia aggregate and confluent. This habit appears to be due to the restrictive force exerted by the epi-stratum of bark through which or under which the peridia develop.

A flaky, thin and more easily displaced bark on an older portion of the tree would tend to give less resistance to a broader development of the peridia, while a tough, compact and newly-formed bark on younger sections would permit only of isolated eruptions. These would have a tendency to elongate, since they would be restricted from expanding laterally. The leaf traces on the branches seem to be the common points of emergence of the aecia in the younger tissues when the eruption is new. Later the fungus is capable of forcing off sections of the bark and the aecia develop closer together, in many cases becoming confluent. The portion of the type material examined shows several elongated peridia erupting through the leaf traces, with little or no indication that the bark had been split or ruptured in the process. The _P. contorta_ material (fig. 4) exhibits a similar condition, and the blisters on the seedlings of _P. ponderosa_ (fig. 10) likewise exhibit this method of rupture when appearing at the leaf traces. Small openings or areas having little resistance seem to produce the elongated peridia, while the shorter and broader peridia seem to develop from the hemispherical blisters formed under a resistant epi-stratum, or develop from under tough, flaky.

barks on older tissues.

It may be interesting to note here that filaments are to be found in the aecia of other caulicolous rusts not native to this region. The aecia of *Cronartium comptoniae* are found to possess many of these slender processes (fig. 17) within the peridia. One aecium, (confluent) collected in Michigan on *Pinus divaricata,* when examined was found to contain 74 full-length filaments extending from floor to dome. Filaments are rarely present in the aecia of *C. comandrae* Pk. infecting *Pinus contorta* and *P. ponderosa.* If present they are generally centrally located within the peridia, and are stout, cylindrical and tapering toward the roof. The aecia of *C. cerebrum* are found to possess filaments sparingly.

The Influences Causing Variation in Hypertrophy

A question now arises as to the origin of the various forms of hypertrophy apparently caused by the same fungus. These forms have been grouped under two main heads, in order to simplify the comparisons. The spheroid gall type includes all the hypertrophies taking on a spherical shape (figs. 2 and 3). The stalactiform type includes all the remaining forms of hypertrophy taking on shapes other than those approaching spherical galls. These comprise the stalactiform and fusiform hypertrophies (figs. 4, 10, and 11), hypertrophies on main stems and branches showing little or no swelling, but upon examination disclosing distinct enlargement of the annual rings in comparison to uninfected tissues (fig. 10). The "hip canker" forms (fig. 7) upon close examination are found to be merely old infections on the main stems of trees in which the earlier infected areas have died out and the fungus has continued fruiting about this dead and cankered area, gradually invading the nearby uninfected tissues. These infected areas when sectioned show distinctly the hypertrophied annual rings invaded by the mycelium, often assuming the spherical gall form. The simple eruptions on the stems and branches classed as P. stalactiforme and showing no visible swelling, upon sectioning disclose a distinct though slight hypertrophy of the invaded tissues. The type of injury found on the two-year-old yellow pine seedlings at the nursery at Haugan, Montana, in a great number of cases showed visible swellings, and in a few cases galls approaching the spheroid type were collected. The remaining infected seedlings showed no distinctly visible swellings. Upon sectioning the stems, however, the excentric and hypertrophied growth of the tissues, due to the presence of the rust hyphae, was disclosed. Out of 34 infected seedlings sectioned and examined, all showed distinctly, in varying degree, the abnormal development caused by the fungus. Examination of a portion of the type material of P. filamentosum proves the tissues in the twigs bearing the aecia to be slightly hypertrophied on the side where the peridia developed, but no distinct swelling is visible on the surface of the twig. The age of the twig indicated by the annual rings is judged to be about 7 or 8 years, and the infection first produced noticeable changes in the tissues in the sixth or seventh year of its growth. These observations show that a variation in degree of hypertrophy exists between these various forms of infection, ranging from slight enlargements of the infected annual rings causing no noticeable swelling to excessive enlargements of the infected tissues resulting in large spheroid galls. It has been observed that many of the galls formed on P. contorta and P. ponderosa have a tendency to stimulate adventitious tissues and produce one to several small upright shoots issuing from the gall or the adjacent tissues (fig. 18). In several cases where these miniature witches'-brooms were formed, it was observed that the larger shoot bore the typical stalactiform type of the rust at its base, which arose from the spherical gall. The infection on both the shoot and the gall were produced by the same fungus, yet in one case a spherical gall-like hypertrophy was produced and in the other only a slight hypertrophy was evident. This, it seems, bears out the theory that the condition of the tissues infected is responsible largely for the resultant form of the hypertrophy and may be a question of available food supply. The older tissues when infected appear to develop with little or no hypertrophy,and the infection covers considerable longitudinal area along the stem or branch, as, for example, the typical stalactiform type on young branches of older trees and in certain cases young stems. Seedlings up to a certain age develop only the slightly hypertrophied or semi-gall type. Seedlings six years and older more frequently develop spherical galls. Galls appear more frequently in older trees, except in the case of young stands of lodgepole where the "hip canker" is very common, and here again the gall form is originally responsible in forming the "hip canker." The "hip canker" form is to be traced to a typical gall formation, for the wood is found hypertrophied and infected similarly to that of the gall forms. The gall forms are almost invariably found with the hypertrophy affecting the annual rings as far back as the pith. A cross section of a gall shows the infected portion in plain contrast to the uninfected, and the infected tissues appear as two conical areas with the points toward the center flaring out toward the circumference of the two halves of the gall (fig. 19). In such cases the fungus invades the sapwood, causing hypertrophy, and leaves evidence of its sphere of influence in the heartwood. Many of the galls are found to be pear-shaped, with the smaller end toward the tip of the twig (fig. 20). This indicates an arrest of the food supply as it enters the gall and allows of greater growth in this portion, with a correspondingly arrested growth at the end of the gall farthest removed from the food source.

It may be that the correct solution of the problem lies in the fact that this fungus develops from year to year upon the identical infected areas, and that the blister and stalactiform types are but the first year eruptions and cause little hypertrophy. Each succeeding year, then, sees the gall forming and enlarging, due to the reaction between the sap tissues and the fungus. This would hold were it not for the fact that many spherical galls are found which have never fruited, and consequently disproves the theory that consecutive years of fruiting by the fungus are necessary to produce a gall. A gall when cut in half longitudinally shows the consecutive layers of hypertrophied tissues dating back to the annual ring forming at the time infection took place (figs. 19 and 20). In this way it is possible to count back by annual rings and determine the time of infection, or at least the year following infection. On galls which have never ruptured, examination of the annual rings shows that the hypertrophy has been in progress from one to three years.

A majority of the large number of spheroid galls sectioned and examined disclose the first annual ring formed on the branch to be the tissues first affected by the fungus. This would indicate infection on the youngest shoots and coincides with finding spheroid galls more malachite green to one part of acid fuchsin, both 1 per cent solutions in 30 per cent alcohol. The surplus stain is removed on filter paper, the sections washed in 95 per cent alcohol for from 5 - 10 minutes, and then transferred to 70 per cent acid alcohol until properly differentiated. When the differentiation is satisfactory, the sections are placed in absolute alcohol for about two minutes, to completely dehydrate them. They are then transferred to a carbol-turpentine-cedar-oil cleaner for from five to ten minutes. Zylol for ten minutes is followed by mounting in balsam.

Colley[5], in a recent article, has given a clear outline of the staining technique used by him in diagnosing the mycelium of the white-pine blister-rust. This method is given here in outline:

1) Cut specimens into blocks of wood 1/8 to 1/4 inch on side.

2) Soak blocks in 10 per cent aqueous solution of gum-arabic containing 0.5 per cent phenol.

3) Cut sections 15-20μ thick on freezing microtome.

4) Rinse sections several times and drain.

5) Add generous amount of safranin. 2-4 hours.

6) Do not let sections dry. Add lichtgruen. Allow to soak 1 minute.

common on younger branches and on seedlings. Many of the spheroid galls sectioned and examined at the time of their first rupture show plainly that the fungus has been present for at least two years previous. Since the hypertrophy does not become visible until the year following infection, it is evident that from three to five years elapse in some instances from the time of infection to the time the first aecia erupt. In contrast to this condition the stalactiform and lesser hypertrophied forms, excepting the yellow pine seedlings at Haugan, on examination show the malformation extending from two to several annual rings inward from the bark (fig. 20), but in none of these cases could the hypertrophy be traced as far back as the annual ring first formed. This would indicate that the spheroid gall forms and seedling infections are produced from infections originating on young meristematic tissue, while the other less pronounced hypertrophies apparently are produced from infections originating on the older twigs and branches. Goebel[10] states that "the more complex a gall the earlier in the life of the tissue infected must the stimulus be applied." This may have a bearing upon the problem before us, and galls may result from all infections taking place on tissues capable of reacting to a high degree, while the slightly hypertrophied infections may be the result of infection on older tissues limited in their reaction to such stimuli.

In the case of the spheroid galls, the life history of the fungus on the coniferous host is somewhat as follows: A new shoot of the tree becomes infected in the summer months. About July the following year a distinct hypertrophy develops and assumes a spheroid shape. The succeeding year, as soon as temperatures allow, about May or June, pycnia on certain portions and aecia on other portions, appear upon the gall. This gives a period of approximately 24 months from the time of infection to the time aecia develop. In the case of the fusiform hypertrophies on the 2-0 yellow pine seedlings found infected at Haugan, Montana, the seedlings became infected about June of the first year of their growth. The following May aecia erupted from the hypertrophied areas. This gives a period of development of but 12 months for this type in contrast to 24 months for the other. What is the explanation for this variation if the fungus causing each hypertrophy is identical? In the first place, the conditions surrounding the infected seedlings favored the rapid development of the fungus. The seedlings were overcrowded and weakened, and by overcrowding formed an ideal culture area for the fungus. In the second place, the fungous hyphae were in the direct path of all the nourishment manufactured by the young host, and received their full share of food, thus stimulating an early fruiting. Soft bark tissues and the moist condition of the air layer just beneath the densely crowded seedling tops would undoubtedly favor the early eruption of the aecial pustules. On the other hand, no such favorable conditions surrounded the development of the fungus infecting a young twig on a tree exposed to drying winds and sunlight. The available food supply in the twig would undoubtedly be less in comparison, and the drier, tougher, bark would be more resistant to rupturing.

Hedgcock, Bethel, and Hunt[22a] have come to the decision that "Peridermium filamentosum and Peridermium harknessii, although both have their uredinial and telial forms on Castilleja (C. coleosporioides) are considered to be either distinct races of the same fungus, or, what is more probable, distinct species." The above article is given as an abstract and no cultural or other proof accompanies it.

MORPHOLOGY OF THE HYPHAE OF THE TWO FORMS OF HYPERTROPHY

Morphology of the Gall Type

An important part of the study of the mycelium within the infected tissues is the technique necessary in properly differentiating the fungous tissues from those of the host. Boyce[3] has recently assembled the scattered information concerning the imbedding and staining of diseased wood. The following method, chiefly taken from Boyce, is one of the best:

The procedure is as follows: - The infected wood is cut into blocks of suitable size for clamping directly into a sliding microtome. The blocks are then heated in a mixture of three parts of 95 per cent alcohol, three parts of distilled water, and one part of glycerine. To soften the wood, the mixture is kept just below the boiling point for from fifteen to twenty minutes. The blocks are then sectioned into 95 per cent alcohol, and then stained for one hour in a mixture of nineteen parts of

7) Remove green stain. Add absolute alcohol and shake in watch glass. Repeat.

8) Replace alcohol with clove oil.

9) Replace clove oil by zylol after placing on slide. With this method the parasitic hyphae stain green to greenish-pink, and the nuclei are red. The host cytoplasm and cellulose walls stain green.

The above method was used to a considerable extent in the study of the stalactiform type, and gave good results.

The radial sections taken from the gall produced on P. contorta were first examined and notes made on the morphology of the hyphae within the tissues. Free-hand sections were cut, some were stained with eosin, some with malachite green, and some by Durand's method.[7] The easin-stained sections afforded good material for the study of the penetration of the fungous tissue into the host. These sections showed the mycelium thickly matted just below the aecium and forming continuous masses, often forcing the parenchymatous cells wide apart. These masses were most noticeable in the live bark tissues, and no doubt account for the spongy texture of the infected tissues when alive. From the parenchymatous tissue toward the wood, the hyphae take on a less massed appearance, and develop into groups and strands, the latter extending into the medullary rays and the pitted cells of the wood. The pitted cells show wavy and contorted in mass (fig. 19), and occasionally the pits are arranged in two rows alternating instead of in single rows. This has been noted by Stewart[31] in his study of the anatomy of the gall produced by <u>Cronartium cerebrum</u> Pk. (H. & L.). Tubeuf[32] and Hartig[13] find the mycelium of Peridermium pine living intercellularly in the rind, bast and wood of its host. It extends haustoria into the living cells, the parenchymatous tissues being those principally attacked. The mycelium penetrates along the medullary rays for a considerable distance into the wood, and the attacked cells lose their cell contents and produce turpentine (resin). Tubeuf states that when the mycelium grows among the dividing cambium cells, these cells are killed.

In the study of the sections cut from the gall type it was found that the hyphae extended along the medullary rays beyond the darkened area (where hypertrophy first is noticeable) up as far as the pith.

The hyphae near the pith were not so numerous as in the tissues showing hypertrophy. The hyphae appeared to be more numerous in the gall sections than in the other type of infection, and were found to measure from 3.5 μ to 5 μ in diameter. The haustoria were very common in the parenchyma cells, ranging from 5.5 to 10 μ in diameter, and from 12 to 24 μ in length. The shapes taken by the haustoria vary greatly, but in all cases noted constrictions were very apparent at the point of entrance into the cell. The haustoria were found to be uninucleate.

It is significant in the case of the gall type that brooming occurs only in connection with the globoid hypertrophy (fig. 18). Of the two forms, the gall type shows plainly a distinct hypertrophy both in the study of the gross specimens and in the study of the microscopic sections. In the latter the wavy and distorted pitted cells, and the unusual abundance of medullary rays, attest to the stimulus exerted by the presence of the hyphae. Where brooming occurs in connection with the galls we have two types of malformations, evidently caused by the same parasite, the gall and the witches'-broom. Undoubtedly the gall is produced on the part of the host where embryonal tissues are present, and especially in the region where buds are forming. The presence of the hyphae in or near the vegetative buds produces the abnormal shoots termed "witches'-brooms"[10]. These shoots are always negatively geotropic and sterile, and are common on other hosts as a result of parasitic fungous infection.

Morphology of the Hyphae of the Stalactiform Type

So far as studied it is found that the hyphae of the stalactiform type of hypertrophy do not vary greatly in morphological characteristics from those of the gall type, although examination of the stained sections shows a different habit of attack. The hyphae are not found in such dense masses just below the aecia, or in the parenchymatous tissues, as is the case in the gall type. The parenchyma cells are rarely forced wide apart, but appear to suffer directly from the attack, as evidenced by the broken-down cell walls and the occupation of the entire cell cavity by hyphae. The larger masses of hyphae are found in the live bark tissues, and from these issue long strands which penetrate the wood along the medullary rays. Hyphae are found beyond the discolored areas. These areas act as indicators of the year infection takes place.

The haustoria appear little different from those found in the gall sections, being slightly larger and more irregular in shape.

Slight hypertrophy of the affected part of the host is apparent in the case of the Stalactiform type, but no hypertrophy of cells and individual tissues is apparent as in the case of the gall type. The pitted cells and medullary rays appear normal. The pitted cells do not appear wavy and contorted in mass, and there are present no unusual number of rays. In the gall sections the medullary raye were unusually abundant in the hypertrophied areas. So far no brooming has been observed in connection with the Stalactiform type, although the infection has often been observed on portions of the host where buds were issuing. In many cases the infection has been found to spread to the young twigs issning from the main infected stem (figs. 4 and 11).

Identification of the Fungous Hyphae in the Host Tissues

The matter of the identification of Cronartium coleosporioides in the host tissues of Pinus is a comparatively simple one when the characteristics of the mycelium are studied. The rusts are characterized by branched and septate mycelia, ramifying in the walls of the cells and extending haustoria into the cell cavities. Colley[5] states that "The Peronsporales and the Erisyphaceae are the only other groups of parasitic fungi besides the Uredinales which possess prominent haustoria." This fact makes identification easier as far as the pine hosts are concerned.

The characteristic traits of the mycelium of C. coleosporioides in either form of hypertrophy on pines are as follows: (1) Comparatively large size and uniform diameter, (2) haustoria, (3) intercellular position of hyphae, (4) forcing apart of parenchymatous cells, and, (5) uninucleate hyphae. The hyphae range in diameter from 3.5 to 5 μ and average about 4 μ. They are constricted sharply where the haustoria enter the cell cavity and expand into irregular shapes within the cavity. The haustoria are common in the parenchyma cells of the cortex, but are not found penetrating the true wood cells. They are large in diameter compared with the hyphae, ranging from 5.5 to 10 μ in diameter and 12 to 24 μ in length. Fig. 21 shows the characteristic shapes of the haustoria within the parenchyma cells of the bark. They are all uninucleate.

The hyphae occupy an intercellular position within the tissues, often forcing the cells widely apart. This is especially noticeable in the tissues just beneath the aecium where the cells are greatly separated and the spaces between filled with a thick matting of hyphae. The hyphae are all uninucleate except those occurring just below the aecium. Those hyphae composing the so-called basidium are binucleate as a result of the sexual fusion of two cells, and this stage indicates the transition from the uninucleate to the binucleate condition.

METHODS OF INFECTION

Infection of Herbaceous Hosts

In considering the various ways in which the germ tubes of the aeciospores may enter the protective tissues of its hosts, it will be well to remember that some opening, either natural or artificial, must be present in the protective coat, in order that the germ tube may penetrate. In the case of fungi which attack the leaves of plants, it is found that the stomata almost invariably furnish the desired openings for the entrance of the germ tube. In some fungi the germ tube has the power to penetrate the cell walls directly. Recent observations show that the sporidia of Melampsora medusae enter the needles of Larix through the stomata. Other instances of penetration of the germ tubes of rust spores by way of the stomata are too numerous to mention. Recently, Fromme[8], by means of a series of experiments, demonstrated that light plays an important part in directing the germ tube over the surface of its host and ultimately aids in the penetration of the host by way of the stomata. This negative heliotropism has never been demonstrated for aeciospores of *Cronartium coleosporioides*. Robinson[29], experimenting with aeciospore germ tubes of *Puccinia poarum*, found them to be indifferent to the action of light. In experimenting with the germ tubes of the sporidia of *Puccinia malvacearum* Mont., Robinson[29] found that they grew away from the light, and Mains[24] states that Fromme reported the same for the germ tubes of the uredospores of *Puccinia coronata* Corda. Another apparent factor recently demonstrated by Graves[11] is the negative chemotropic reaction of germ tubes of such fungi as *Rhizopus nigricans* toward the metabolic products of their hyphae. Ward[37] and Robinson[29], in experimenting with decoctions of the host, found these had no appreciable effect upon the germination of the spores tested. Attempts were made to test host decoctions upon spores of *C. coleosporioides*. Spores from the uredinial eruptions on leaves of Castilleja were sown in yellow pine decoctions and in Castilleja decoctions. No germination was recorded for those spores in the yellow pine liquid, while there was slight germination for those in the Castilleja decoction. Aeciospores of the same fungus taken from eruptions on 2-year-old seedlings of yellow pine, when sown in hanging drop cultures of Castilleja decoctions, gave copious germination in all three trials made (fig. 22). Similar results were secured from aeciospores from the "hip canker" of lodgepole pine sown in hanging drop cultures of Castilleja decoction. Controls sown in distilled water gave very little germination. From these preliminary experiments the correct host decoction apparently has a definite chemotropic effect upon the germination of the spores.

Examinations of Castilleja leaves shortly after inoculation with aeciospores of _C. coleosporioides_ (stalactiform type) disclose the fact that stomatal openings are the main entrance points for the germ tubes.

Infection of Pines

Other than stomatal openings, the leaf traces, freshly formed by the dropping off of needles on young portions of the pine host, no doubt play an important part as openings for the germ tubes of the sporidia (fig. 23). Insect punctures and other mechanical injuries are also possibilities to be considered, although in injuries where much resin is caused to flow, the chances for infection are not so good. A peculiar phase of cultural work with teliospores on various species of pine is the repeated attempts to secure successful inoculations upon various pines. Teliospores from infected Castillejas in the field and from Castillejas infected artificially in the greenhouse were tried, as well as aeciospores taken from the aecial eruptions on pines, but none of these produced infection. Little opportunity was had to observe the penetration of the sporidia germ tubes, and this remains for future observation.

Possible Parasitism of Castilleja Species on Roots of the Plants

The peculiar action of Castilleja plants when transplanted from the field to pots in the greenhouse has raised the question of the possible parasitism of Castilleja species upon the roots of other plants. Nearly all the transplanted plants have failed to continue alive over one season. A few have revived the following spring, but never attained normal size, remaining weak and etiolated. In nature, vigorous plants of certain species are produced annually from the same root stalk. Two small, etiolated shoots of Castilleja miniata developed (in March, 1918) from underground root stalks left in pots since the spring of 1917 in the greenhouse at Missoula, Montana. In May and June of 1917 these pots contained normal, flourishing plants, some of which were used in inoculation experiments. The undeveloped shoots of 1918 will no doubt react as others have done before, finally wilting and dying before maturity.

Species of Comandra, alternate hosts for Cronartium comandrae Pk., have been found to parasitise a large number of plants[21]. The roots of Comandra were found attached to the roots of the host plants by means of hemispherical disks of holdfasts.

The close association of the Scrophulariaceae with the Orobanchaceae, which are parasitic on roots of other plants, may be significant in respect to the possible parasitism of the members of the former family. The few observations made upon this genus and upon species of Castilleja, have so far disclosed no connection between these plants and the roots of neighboring plants.

THE RELATION OF OTHER FORMS OF LIFE TO THE RUST

Fungi

Other forms of life enter into the biology of this pine rust and each form plays a definite part in its life history. Beginning with the lower forms of life, fungi are found to be the first organisms living in definite relationship to the rust. Tuberculina maxima Rostr. is found occasionally attacking the pycnial and aecial stages, forming lilac-colored, powdery masses along the bark crevices and open eruptions of infected areas. This powdery mass is composed of countless numbers of lilac to nigrosin-colored, ellipsoid spores (fig.24) borne on pedicels. This fungus parasitizes the rust attacking the stromatal layer as well as the fruiting bodies, and in some cases develops sufficiently to check the progress of the rust on the pine host. It occurs less frequently upon Cronartium coleosporioides than upon C. cerebrum and C. comandrae, and is found most frequently upon the latter. Its economic importance in checking the rust so far as this region is concerned is not very great[36], although detailed studies might yield interesting data.

Mistletoe

The false or dwarf mistletoes (Razoumofskya spp.) of the Rocky Mountain region are common parasites on the various native conifors. These parasites attack the trunk, branches, and twigs of the host, causing "witches'-brooms" and in many cases large swellings of the attacked part. Some of the smaller swellings on branches and on stems of young trees have been found with fresh eruptions of C. coleosporioides on them. The mistletoe infections on some of these were determined to be several years old, which makes it evident that the rust infection was secondary. Many cases are found where the two parasites grow in close proximity on the same branch (fig. 25) and in a great number of them the mistletoe was found to have preceded the rust infection. Cases are common also where it is evident that the rust infection was followed by that of the mistletoe. It is possible that the effect upon that portion of the host attacked and the openings presented by either of these parasites furnishes favorable conditions for whichever one is secondary in securing a foothold upon the host.

Razoumofskya americana (Nutt.) Kuntze on *P. contorta* and *R. campylopoda* (Engelm.) Piper on *P. ponderosa* are the two principal species of dwarf mistletoe which are found in connection with the rust here discussed.

Insects

In the study of the relation of insects to the life history of this rust, especially that portion of it dealing with spore distribution, there lies a wide and virtually unexplored field. The rust, distributed over a wide territory and developing in an environment favorable to many insects and possessing a stage (pycnia) which by its sweet fluid undoubtedly attracts certain of them, lends many opportunities for complex relations. It would seem strange if no relationships existed between these two forms of life. Field observations begun in 1914 and continued since then have resulted in establishing a certain relationship between ants, aphids, and the pycnial and aecial stages of the rust. In the Deerlodge National Forest in 1914 a certain species of ant (Lasius niger var. americanus) was found to be very common in the lodgepole pine (P. contorta) stands. This species infested the cankered and ruptured areas on the lodgepole pine, caused by C. coloesporioides. Upon closer examination of one of the trees the small crevices in the bark and cankered areas were found crowded with a dark-colored aphid. These were especially abundant on areas developing pycnia and were also distributed among the aecial eruptions. The ants visited these aphids continuously, securing their honey dew, and in so doing accumulated some of the aeciospores which they carried away on portions of their body. Those ants returning from the aphid colonies moved down the tree trunk and some were observed heading for an anthill some few feet away. Castilleja plants were abundant in this vicinity, and were also found heavily infested with a small green aphid. The same species of ant were found moving about these aphid colonies. A few of these ants brought to the laboratory for examination disclosed aeciospores of the rust clinging to their bodies. Here may be found an interesting means of spore distribution almost direct from one host to the other, and, with the punctures made in the leaves of the Castilleja by the aphids, a ready means of entrance afforded the germ tube of an aeciospore. Many other observations in different regions have resulted in finding similar conditions. Large colonies of ants and aphids were found infesting the lesions of the rust on lodgepole pines near Haugan, Montana, and here some of the ants examined were almost yellow with the spore dust.

Often in the collections of the rust brought to the laboratory several small grayish-white grubs were found burrowing among the erupted aecia or under the bark flakes among the unruptured tissues. In time the surface of the gall or lesion is eaten bare, peridia and spores both disappear, and only a grayish refuse is left. A few of these larvae were preserved alive for a period of time, and eventually developed into small chestnut-brown beetles about 3½ mm. long, of the family Dermestideae. These beetles were sent to R. W. Wells at the State College at Bozeman, for determination. They were finally determined as Epurea ovata Rand, by H. F. Wickersham of Iowa. These larvae were found on all forms of the rust on both P. contorta and P. ponderosa, and necessitated the early application of napthalene to the specimens, in order to save them from destruction. Such entire destruction of aecia and aeciospores along with portions of the stromatal layers no doubt checks the development and spread of this fungus in the field.

Another insect found inhabiting the pine hosts common to this fungus is Evetria albicapitana Busch, a species belonging to the order Lepidoptera. The larva of this moth forms large pitch tubes on the twigs, branches and stems of pines resulting from its tunneling activities in the bark. These tubes form possible infection wounds for the entrance of the rust, and the bright yellow aecial eruptions are often found associated with these insect pitch tubes, indicating a close relationship between the insect injury and the rust infection.

Animals

Of the animals found associated with the life history of the rust the rodents are by far the most prominent[34]. By firtue of their life habits, necessitating a thorough inspection for seed cones of a large number of the species of trees acting as hosts to the rust under discussion, the Western pine squirrels (Sciurus richardsonii Buchman) are the most important in this respect. It is a common observation during the early spring, or at the time the perennial mycelium of the rust gall is maturing its countless spores, to find the bark of galls and lesser swellings eaten away by rodents (fig. 26). Before the aecial spores are mature and also during maturity, a sweet-tasting liquid is exhuded by the fungus, which contains the pycniospores and which seems to be very attractive to certain of these animals. Until recently no clew of the particular animal or animals feeding on the pycnial and aecial stroma was obtainable. Several instances of the capture of the common Western pine squirrel actually engaged in gnawing the galls have furnished the necessary evidence. A careful examination of these animals showed the fur of the head and body to be thoroughly dusted with the spores of the fungus. A similar examination of the contents of the stomach revealed the presence of the spores in great numbers mixed with the partially masticated inner bark of the gall.

These ground squirrels have been observed to climb some three to four feet from the ground into brooms caused by the rust. The infected areas on the young broomed trees were afterward found to have been freshly gnawed. This habit is unusual for this species of rodent. In thin, open stands of young yellow and lodgepole pines in many localities of the Northwest various species of pine rusts are very abundant and do great damage to young reproduction. It is quite possible for the squirrels, since they travel much over the ground, to aid in the spread of these diseases by carrying the spores from the infections on the pines to the alternate host.

On the Deerlodge National Forest porcupines do a large amount of damage in peeling the bark of the lodgepole pine (Pinus contorta Loud.), attacking the main stem as well as the branches. A large number of these injuries were found to have been made on lesions of the trunk and branches caused by Cronartium coleosporioides.

The Columbian ground squirrel (Spermophilus columbianus Ord.) is also known to use the bark of the galls for food. There here arises a question whether this gnawing of infections and consequent spore-carrying is a benefit or an injury. Such a question can not be answered off-hand, as no definite figures are available to determine the extent to which the carrying of spores by squirrels extends the distribution and development of the fungus. In most cases the gnawing away of infected bark tissues can be reckoned as a benefit in so far as removing possible spore-producing fungous tissue is concerned. Galls, when uniformly gnawed (fig. 26) invariably die[34]. Lesions other than of the gall type also dry out and fail at a revival of the fungus after a rodent has painstakingly gnawed away all the soft, infected tissues. In many cases, where the infection has extended completely around branches or even main stems and has been entirely eaten away, a girdling of the branch or tree results. On an area of approximately three acres near Hayden Lake, Idaho, the lodgepole pines were found heavily infected with the P. stalactiforme type of the rust. Out of this area 45 trees ranging from three to twelve inches D. B. H. were gnawed as far up and down the trunk as the infections extended, these ranging from three to fifteen feet.

A particular tree observed in the Deerlodge National Forest where the rust is very common on the lodgepole pine, was found to have been killed by girdling due to rodents. A large area about the lower trunk, several smaller areas on the upper trunk and numerous lesion and gall infections on branches were entirely gnawed away, exposing the whitened wood. The large, band-like area about the lower trunk still showed evidences along its edges of the presence of the fungus.

Infections on pines caused by C. coleosporioides are not the only ones attracting rodents. The lesions formed on P. contorta and P. ponderosa by C. comandrae are especially prized by pine squirrels, who in their thoroughness in removing all portions of the infected tissues often girdle valuable trees. On an area of approximately two acres above Bonner, Montana, is growing a young stand of P. ponderosa. These trees were heavily infected with C. comandrae, principally along the main stems, and in 1916 the squirrels girdled nearly a fourth of the infected trees by gnawing away the soft, corky tissues before the aecial eruptions were due. Squirrels are very adept in recognizing newly infected areas of C. coleosporioides, or, in the case of C. comandrae, the newly infected tissues just beyond the previous year's eruptions. The gnawing of these areas is generally done in the very late winter, or early spring before the aecia erupt. No doubt the presence of the sweetened pycnial layers has something to do with the choice.

DAMAGE AND CONTROL

In order to secure more accurate and definite knowledge concerning the amount and kind of damage this pine rust causes, a few surveys were made of infected areas. These are given in Tables I to V, inclusive. Tables I to III represent areas of infection of lodgepole pine, while Tables IV and V show the effect of the fungus upon Western yellow pine.

It will be noted, the first four tables indicate that the reproduction is seriously infected by the fungus, and that a considerable number of these trees are found to have died from the attack. In Tables I, II, and III the percentage of dead trees to infected trees is 8, 7, and 12, respectively. In Table I, 38 per cent of the total trees on the area were found infected. Of this total number of trees, 398 were of the reproduction size up to 6 inches D. B. H., and 90 were trees between 6 and 16 inches D. B. H. All of the infections recorded were either fusiform or globoid in shape, and of the total infections, 60 per cent were found upon branches. The percentage of infected trees in Table II is 40, and in Table III, 51. In the latter table it is seen that 73 per cent of the total number of infections were found upon the trunk. In all cases the reproduction is seriously damaged, and in Table III one of the larger trees is recorded as dying from the effects of heavy infection by the rust.

In Table IV, 45 per cent of the total number of trees on the area were found infected, and 61 per cent of the total infections were found upon the trunks. The percentage of infected trees dead from the attack is 11, with the reproduction suffering the majority of loss, and 40 per cent of the total branches infected were found to be dead as a result of the numerous infections. The reproduction on this area suffered severely, and indications are that few will survive. In all cases where the larger trees are heavily infected, a stunted growth results, due to loss of crown caused by the death and stunting (figs. 18 and 27) of numerous branches.

e. I.--Showing Effect of Cronartium coleosporioides on Pinus contorta.

,-1 A. Date of Survey: March 28-31, 1916. Locality: Coeur d'Alene, Ida.

Uninfected trees	Infected trees	Total No. of galls, fusiform and globoid		Total No. of lesions		Alternate hosts	No. of trees dead from attack	No. of branches dead from attack	Estimated injury or cull.
		Trunk	Branch	T.	B.	Name and amt.			
263	135	134	170			Castilleja lineata abundant	16		Most of reproduction will probably die.
40	50	35	84			Do.		20	Stunted growth
303	185	169	254				16	20	
62%	38%	40%	60%				8% of infected trees		

e 17.

e II.--

½ A. Date of Survey: July 21, 1916.

Uninfected trees	Infected trees	Total No. of galls, fusiform and globoid: Trunk	Branch	Total No. of lesions: T.	B.	Alternate hosts: Name and amt.	No. of trees dead from attack	No. of branches dead from attack	Estimated injury of cull.
209	150					Castilleja lineata; over 200 plants on area found infected with C. coleosporioides	11		
4						Do.			
213 60%	150 40%						11 7% of infected trees.		

e III.--

½ A. Date of Survey: March 28-31, 1916.

Uninfected trees	Infected trees	Total No. of galls, fusiform and globoid: Trunk	Branch	Total No. of lesions: T.	B.	Alternate hosts: Name and amt.	No. of trees dead from attack	No. of branches dead from attack	Estimated injury of cull.
330	353	338	113	4	1	Castilleja lineata; very abundant over entire area.	42		Reproduction infected beyond recovery. Many still live; have yellow foliage
22	4	2	8			Do.	1 dying		Stunted growth
352 49%	357 51%	340 73%	121 27%	4 80	1 20%		43 12% of infected trees		

ble IV.--Showing Effect of Cronartium coleosporioides on Pinus ponderosa. Area, # A. Date of Survey: March 28-31, 1916.
ocality: Coeur d'Alene, Idaho.

D. B. H.	Uninfected trees	Infected trees	Total No. of galls, fusiform and globoid		Alternate hosts	No. of trees dead from attack	No. of branches dead from attack	Estimated injury or cull
ches	No.	No.	Trunk No.	Branch No.	Name and amount			
pro- o- on	95	73	114	68	Castilleja lineata in abundance.	9	40%	Most will die.
6	12	13	11	12	Do.	1	40%	Stunted growth
als:	107 55%	86 45%	125 61%	80 39%		10 11% of infected trees	40%	

le V.--A Study of One Tree of Pinus Ponderosa, 110 Years Old, 120 Feet gh. Date of Survey: April 1-4, 1916. An Average Tree of the Stand.

D. B. H.	Uninfected trees	Infected trees	Total No. of galls, fusiform and globoid		Alternate hosts	No. of trees dead from attack	No. of branches dead from attack	Estimated injury or cull
hes	No.	No.	Trunk No.	Branch No.	Name and amount			
4		1		173 20% at base of cones. 50% at internodes 30% at branch whorls	Castilleja lineata in abundance		Ends of most all infected branches dead.	Stunted growth dead branched.

In Table V is given a careful record of a single, heavily infected tree of yellow pine, 110 years old, examined after felling. This tree represented the average of the stand in which it grew, in respect to seriousness of infection, and was 34 inches D. B. H. and 120 feet high. It bore a total number of 173 infections occurring upon the branches. Of these, 20 per cent were found to occur at the base of cones, 50 per cent at the internodes of the branches, and the remaining 30 per cent at the branch whorls. It is recorded that the ends of most all the infected branches were dead.

On all these sample areas Castilleja species were very common, and at the proper season bore heavy infections of the uredinial and telial stages of the rust.

On these sample areas (Tables I, II, III, IV) the percentages of trees dying from infections of the rust range from 7 to 12, and it is safe to say that this is a conservative estimate of the damage done on a majority of the infected areas in this region. In such regions as the Big Hole River basin of the Deerlodge National Forest, and the Grangeville region of the Nesperce National

Forest of Idaho, the lodgepole pine in the former, and the lodgepole and yellow pine in the latter are even more seriously attacked. The "hip canker" form of the rust on lodgepole pine often results in the killing of the infected tree whenever the canker spreads so as to girdle the trunk.

Another form of injury resulting in economic loss is the frequent occurrence of the gall type of infection at the base of cones, particularly of lodgepole pine (figs. 28 and 29). In many cases the infection aborts the cone, or else the nourishment received by the cone is so slight that it never matures. This is important from the viewpoint of seed production.

The control of this disease is difficult, on account of the heteroecious habit of the fungus causing it. If the herbaceous hosts were easier to eliminate, the question of control would be simplified, but their place and manner of growth makes extermination difficult. Eradication of the herbaceous hosts could only be considered economically justifiable in the protection of susceptible seedlings in the forest nurseries. Here again the cost of control, in many cases, may prove prohibitive.

Since it is necessary to destroy the plants for a distance of several hundred feet from the nursery beds, and since it avails but little to merely destroy the aerial portions of the plants and leave the extensive annual root stocks in the soil, the problem becomes a difficult one. Some of the more effective weed-destroying chemicals, such as iron sulphate, copper sulphate or sodium arsenite solutions, might be used to advantage. Grazing the area to animals which use these host plants as food may prove effective, providing the grazing is intensive all through the growing season and the herbage kept quite short, so as to allow a minimum of leaf surface open to infection. In this connection it may be interesting to give the report of C. E. Fleming, who states that cattle and sheep use plants of Castilleja species for food, but only to a limited extent. _Castilleja chromosa_ is quite extensively grazed by sheep.

Spraying the seedlings in the nursery with 4 - 4 - 50 soap-Bordeaux has been used at the Savenac Nursery at Haugan, Montana. Spraying in conjunction with the removal of all infected pine material within a quarter of a mile of the seedling beds has resulted in a marked reduction in the number of infections found on the seedlings.

The problem of control in the forest is even more difficult than in the forest nursery, and the expenditure for control methods is less justifiable, in view of the extensive rather than intensive methods of forestry in present use. Some progress toward bettering the health of the forest can be made, however, by recommending that the timber sale contract clauses, or the marking regulations, stipulate the cutting of all rust-infected trees[39]. Grazing may be found helpful in reducing the amount of herbaceous host material upon an area infected with the rust.

SUMMARY

The distribution and life cycle of the rust Cronartium coleosporioides infecting pines in the Northwestern states is taken up in detail, and a careful review of cultures is given. New hosts for the telial stage of the rust are recorded.

The various forms of hypertrophy caused by the rust and occurring on pines are discussed. It is found that specific differences are to be noted between the gall and the stalactiform type of malformation. These differences are expressed in such factors as the effect of the

of this rust. The list ranges from _Tuberculina maxima_, of the Imperfects, which parasitizes the pycnial and aecial forms of the rust, to animals of the rodent type. The data presented indicate that considerable damage is done to all classes of pine hosts, from seedlings up to veteran trees, by the attack of this rust. The most destructive attacks are those made upon seedlings, especially the very young ones developing in forest nurseries. Killing and dwarfing of young seedlings in the forest are also common, and considerable injury is done the older trees by the formation of galls and the killing of live tissues, so as to produce girdling. Brooming and stunting of leaders and branches, and deformity of cones, are often produced.

Control measures are recommended for both intensive work in forest nurseries and extensive operations in the forests.

The definite answer to the question "Are the two forms of hypertrophy caused by the identical fungus, or are there two distinct species?" can not be answered with certainty until pedigree cultures are made to determine this point. Part of the data presented indicate that the two hypertrophies are caused by the identical fungus having its alternate stage upon Scrophulariaceae. The remaining data point to two distinct species, or at least two distinct races of the same fungus.

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